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The *E.coli fis* promoter is subject to stringent control and autoregulation

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The DNA binding protein FIS is involved in processes like site specific DNA inversion, λ excision and stimulation of stable RNA synthesis in *Escherichia coli*. The amount of FIS protein is subject to dramatic changes during growth. We demonstrate that *fis* is part of an operon with one ORF of unknown function preceding the *fis* gene. Regulation of *fis* synthesis occurs at the transcriptional level. Within 15 min after nutritional upshift a large burst of *fis* mRNA is produced which levels off when cells begin to grow. By mutational analysis using promoter-*lacZ* fusions we demonstrate that the *fis* promoter is autoregulated by FIS. Growth phase regulation of the *fis* promoter depends on the presence of a GC motif downstream of the −10 region. We show that the *fis* promoter is subject to stringent control and discuss this unusual feature with respect to the known and putative functions FIS serves in *E.coli*.

Key words: fis operon/guanosine tetraphosphate/promoter/stringent control/transcriptional control

Introduction

FIS is a small heat stable DNA binding protein in *Escherichia coli* (Johnson *et al.*, 1986; Koch and Kahmann, 1986) which was identified by its ability to stimulate the site specific DNA inversion reactions catalysed by the DNA invertases Gin, Hin and Cin. In these systems the stimulatory effect is mediated by the specific binding of FIS to an enhancer sequence (Johnson and Simon, 1985, 1987; Kahmann *et al.*, 1985; Huber *et al.*, 1985). In binding to the enhancer and bending the DNA, FIS is thought to facilitate the assembly of the synaptic complex either by interacting with the invertase molecules bound to the recombination sites (Bruist *et al.*, 1987; Heichman and Johnson, 1990) or by stabilizing a branch point in DNA which facilitates the formation of the correct synapse (Kanaar *et al.*, 1988). FIS is a dimer in solution and contacts DNA in the major groove (Bruist *et al.*, 1987; Koch and Kahmann, 1986). DNA binding occurs at specific sites which display a quite degenerate consensus sequence as determined by mutational analysis (G/TNNYRNNA/TNNYRNNC/A; Hübner and Arber, 1989). The FIS structure was recently solved by X-ray crystallography. The FIS dimer has a compact structure with four α helices per monomer (Kostrewa *et al.*, 1991). The two most C-terminal α helices constitute a helix-turn-helix DNA binding motif and many mutations in this region cause severe DNA binding defects (Koch *et al.*, 1991; Kostrewa *et al.*, 1991; Osuna *et al.*, 1991). Although FIS is dispensable for growth of the *E.coli* cell (Johnson *et al.*, 1988; Koch *et al.*, 1988) an increasing number of reports implicate FIS in other processes than site specific recombination. FIS has been shown to stimulate stable RNA synthesis in *E.coli* (Nilsson *et al.*, 1990; Ross *et al.*, 1990), to stimulate phage λ excision (Thompson *et al.*, 1987; Ball and Johnson, 1991), to autoregulate its own synthesis (Koch *et al.*, 1991), to modulate phage Mu growth (Bétermier *et al.*, 1989) and to participate in *ori*C regulation (Gille *et al.*, 1991; M.Filutowicz, personal communication). Binding to specific sites appears to be a prerequisite for the observed FIS effects and the ability to change the DNA conformation by bending may be an additional feature required. These systems are distinct from the DNA inversion systems, however, because they do not involve long range effects of FIS.

When studying the occupancy of the FIS binding site in the λ att site by *in vivo* footprinting techniques the interesting observation was made that the occupancy of this site by FIS was subject to drastic variation: while this site was occupied during lytic growth the site was not bound in stationary phase cells (Thompson *et al.*, 1987). This indicated strong changes in the amount of FIS protein during cell growth.

Since growth control in prokaryotes is poorly understood we have investigated this phenomenon with the hope that its understanding might also provide clues to the function FIS serves in *E.coli*. We show that FIS synthesis is transcriptionally controlled. *fis* is cotranscribed with another gene whose function is yet unknown. We demonstrate that autoregulation and stringent regulation are key features in controlling FIS levels in *E.coli*.

Results

Transcriptional control of fis

To analyse *fis* gene expression during growth, RNA of strain CSH50 was prepared at various times after dilution of an overnight culture into fresh dYT medium. The RNA was gel-fractionated for Northern analysis and probed with a DNA fragment from the *fis* gene (Figure 1). Two hybridization signals at 1400 and ~600 nucleotides were visible. The intensity of these signals was very strong in the samples prepared 15 and 30 min after inoculation and decreased significantly at later time points; in stationary cells no *fis* mRNA was detectable. This shows that *fis* synthesis is controlled at the transcriptional level. The half life of *fis* mRNA was determined to be <3 min (data not shown). Therefore the high levels of *fis* mRNA detected during the first 30 min after inoculation reflect high rates of transcription rather than accumulation of transcripts. As the size of the mRNA exceeds the 294 bp *fis* gene this suggests that sequences either 5′ or 3′ of *fis* must be cotranscribed with *fis*. The appearance of two signals could indicate that

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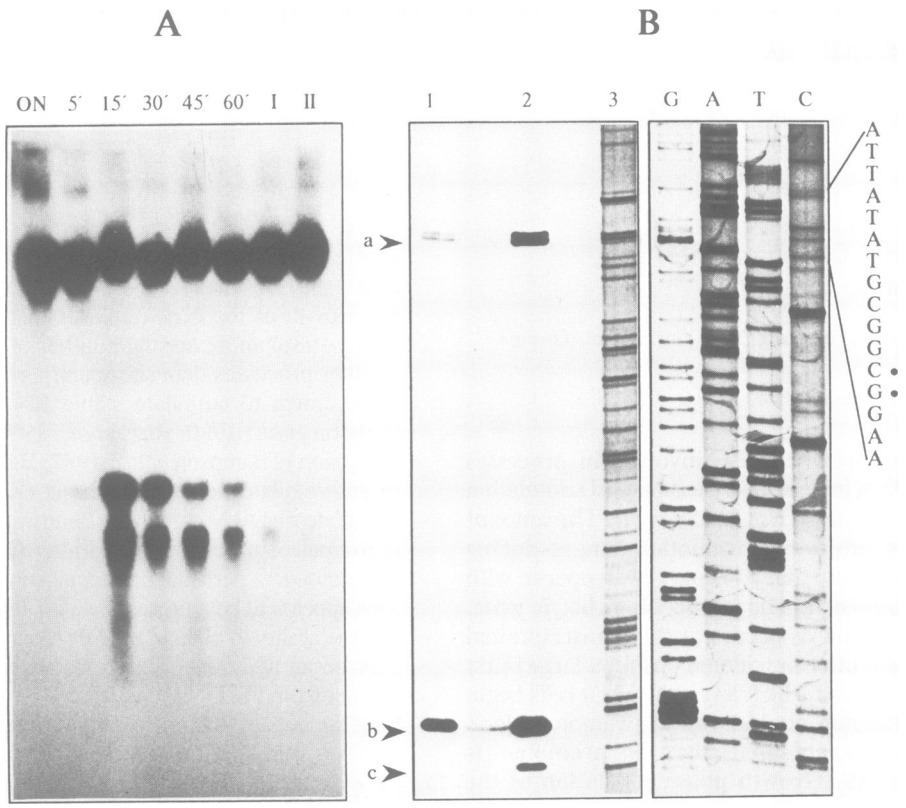

Fig. 1. Northern analysis of *fis* gene expression and mapping of the 5′ end of the *fis* transcript. (**A**) Total RNA was isolated from CSH50 at the indicated times after 30-fold dilution of an overnight culture (ON) in dYT. (I), RNA was isolated from exponentially growing cells at 140 Klett units; (II), RNA was isolated from early stationary phase cells at 260 Klett units. Identical amounts of RNA were loaded on a 1% agarose gel containing formaldehyde (Sambrook *et al.*, 1989). Northern blots were probed with a 690 bp *XmnI*−*NruI* fragment encompassing the coding region of *fis* and upstream sequences. The strong band present in all samples represents chromosomal DNA. Sizes of the two lower bands were estimated as 1.4 and 0.6 kb relative to a 0.2−9.5 RNA ladder from BRL. (**B**) The same RNA as shown in (A, 15′) was used for primer extension using a 50 bp *NcoI*−*HpaII* (see Figure 2) fragment 5′ end-labelled at the *HpaII* site as primer. Extension products were separated on a 6% sequencing gel. (c) marks the primer, (b) the filled in primer and (a) the extension products. In lanes 1 and 2 different amounts were loaded. In lane 3 a sequencing reaction of pTZ18R using the universal primer was run on the same gel. G, A, T and C are sequencing reactions with pCF222 DNA using the labelled *NcoI*−*HpaII* fragment as primer. Alignments with lane 3 were done by running a sample of lane 3 on both gels. The transcription start sites are indicated in the sequence with two dots.

fis is transcribed from two different promoters which are similarly regulated or it could indicate an RNA processing event.

Localization of the fis promoter by primer extension

Using various fragments upstream and downstream of the *fis* coding region as probes in Northern blots we were able to show that the start of the *fis* mRNA maps to a region ~1000 bp upstream of the *fis* gene (not shown). This region was sequenced (Figure 2 and see Materials and methods for details). The analysis revealed the presence of a single open reading frame, termed ORF1, which could encode a protein of 321 amino acids. ORF1 and FIS are encoded on the same DNA strand and constitute a single operon. This was corroborated by the isolation of Tn*5* mutants which had a FIS$^-$ phenotype and mapped either in ORF1 or in *fis* (not shown). A homology search in the gene library using the program Bestfit revealed 59% nucleotide identity over a 500 bp region upstream of *nif*R2 in *Rhodobacter capsulatus* encoding *nif*R3, a putative locus involved in the regulation of nitrogen fixation (Jones and Haselkorn, 1989). Unfortunately no gene has been unambiguously assigned to these sequences (W.Haselkorn, personal communication). Another possibility, namely that the region encoding ORFI does not encode a protein but a stable RNA species seems unlikely because an ORF1 homologue is also found in *Salmonella typhimurium* and shows >90% amino acid identity with ORF1 of *E.coli* (R.Johnson, personal communication).

To map the start of the *fis* mRNA more precisely we performed primer extension experiments (see Materials and methods) with a 50 bp *NcoI*−*HpaII* fragment originating from the N-terminal portion of ORF1 (see Figure 2) and RNA prepared 15 min after nutritional upshift. Two extension products differing by 1 nucleotide in length were obtained (Figure 1B). This places the 5′ end of the *fis* mRNA at a position 33 or 34 bp upstream of ORF1 (see Figure 2). Sequences upstream of the mRNA start have homology to the *E.coli* consensus promoter sequences (Harley and Reynolds, 1987) (Figure 2). Further 5′ to this promoter several sequence features are noteworthy: 6−7 bp long AT stretches are repeated eight times in irregular intervals, a sequence resembling the consensus binding site for IHF (Integration Host Factor; Friedman, 1988) is found at position −81 and two sequences with one mismatch to the FIS consensus binding site are located just upstream of the −35 region and downstream of +13 (Figure 2, I and II). By DNaseI footprinting we could show that FIS indeed binds to these sequences (Figure 3B). Gel retention analysis showed that the *fis* promoter region is bound by FIS with

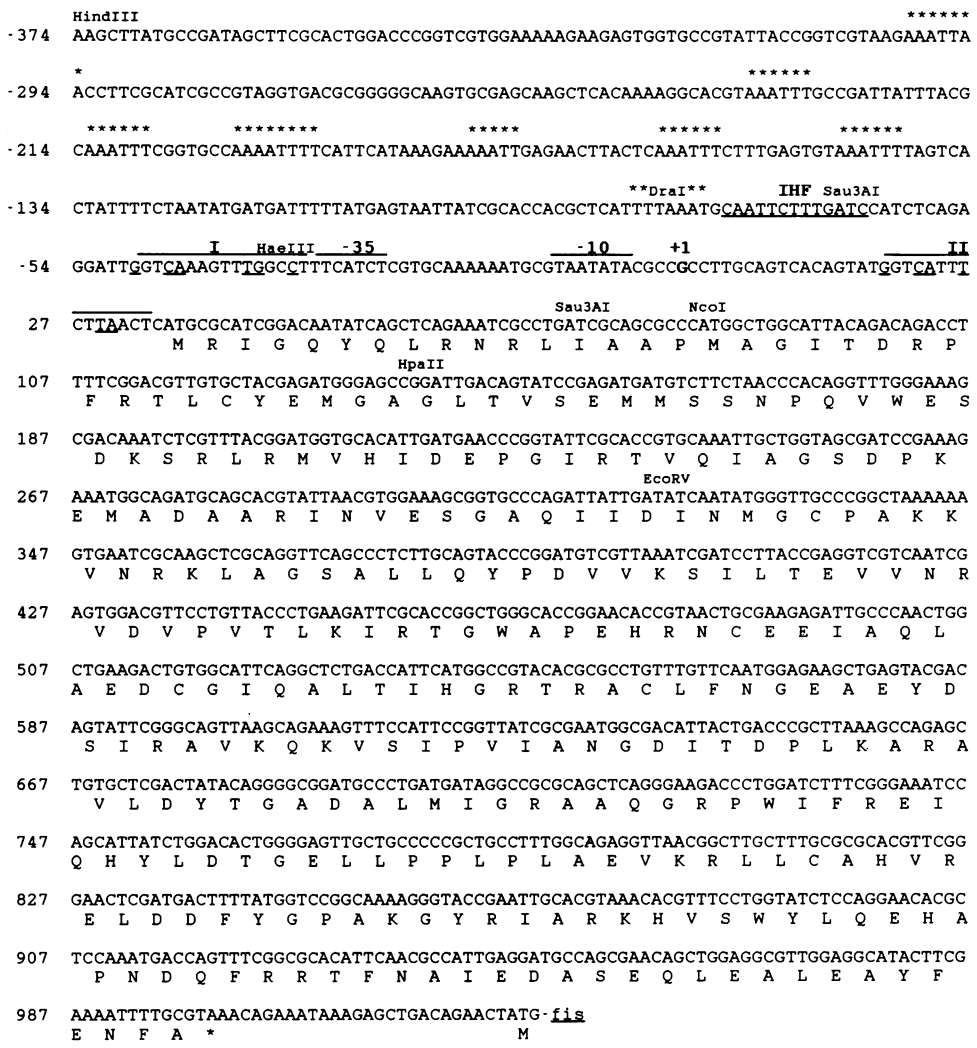

Fig. 2. Nucleotide sequence of the *fis* promoter region and ORFI. The sequence shown extends from a *Hin*dIII site to the start of the *fis* gene (ATG-*fis*). +1 marks the start of the *fis* mRNA. Sequences matching the *E.coli* −35 and −10 consensus are indicated. Asterisks mark the AT-rich sequence motifs, IHF indicates the putative binding site for IHF, I and II mark binding sites for FIS. The FIS consensus sequence (G/T--YR--A/T--YR--C/A; Hübner and Arber, 1989) is found with one mismatch between −49 and −35 in site I and with one mismatch between +19 and +33 in site II. Positions of homology to the FIS consensus sequence are underlined. Only restriction sites used for subcloning are shown.

an affinity comparable with the *rrn*B P1 UAS sequences (not shown).

Regulation of fis gene expression by upstream sequences

To analyse the influence of sequences located upstream of the *fis* promoter on promoter activity we generated a series of promoter−*lac*Z fusions on single copy λ phages in CSH50 and the *fis*⁻ derivative CSH50fis::Kan (see Materials and methods). β-galactosidase activity was determined in overnight cultures of respective lysogens (Figure 3A). In the *fis*⁺ background the highest β-galactosidase levels were measured in λFP1 lysogens where the *fis* operon is fused to *lac*Z at a site in *fis*. The 7-fold reduced β-galactosidase activity observed in strains lysogenic for λFP2 and the additional 4-fold reduction in λFP3 lysogens must be attributed to the different fusion points in the *fis* operon. Northern blot analysis revealed that the 3′ end of the two *fis* mRNA signals in Figure 1A is identical (not shown). Therefore, the promoter downstream sequences in λFP2 and λFP3 most likely contain a RNA processing site or an additional transcription startpoint. Its deletion could lower the amount or the stability of the short mRNA encoding *fis*. We have not studied this phenomenon further. The sequential deletion of sequences 5′ to the promoter in λFP4 and λFP5 lowered promoter activity ~3-fold while the removal of the FIS binding site I in λFP6 led to a 2.5-fold increase in promoter activity. Promoter activity increased further when 3′ sequences encompassing FIS binding site II were deleted in λFP7 (Figure 3A).

When the same *fis*−*lac*Z fusions were assayed in the *fis*⁻ strain promoter activity was 4-fold higher than in the *fis*⁺ strain for constructs λFP2, 3, 4 and 5 while only a 2-fold increase in promoter activity was observed in the strain lysogenic for λFP6. With λFP7 the difference between *fis*⁻ and *fis*⁺ strain was reduced to 1.5 (Figure 3A). The negative effect of FIS on its own transcription was more pronounced when FIS was provided from the multicopy plasmid pCF221. Promoter activity was reduced up to 10-fold in constructs with both FIS binding sites while deletion of FIS binding sites I or I + II resulted in 3.2- and 1.5-fold reductions of promoter activity, respectively (Figure 3A). The decline in promoter activity caused by

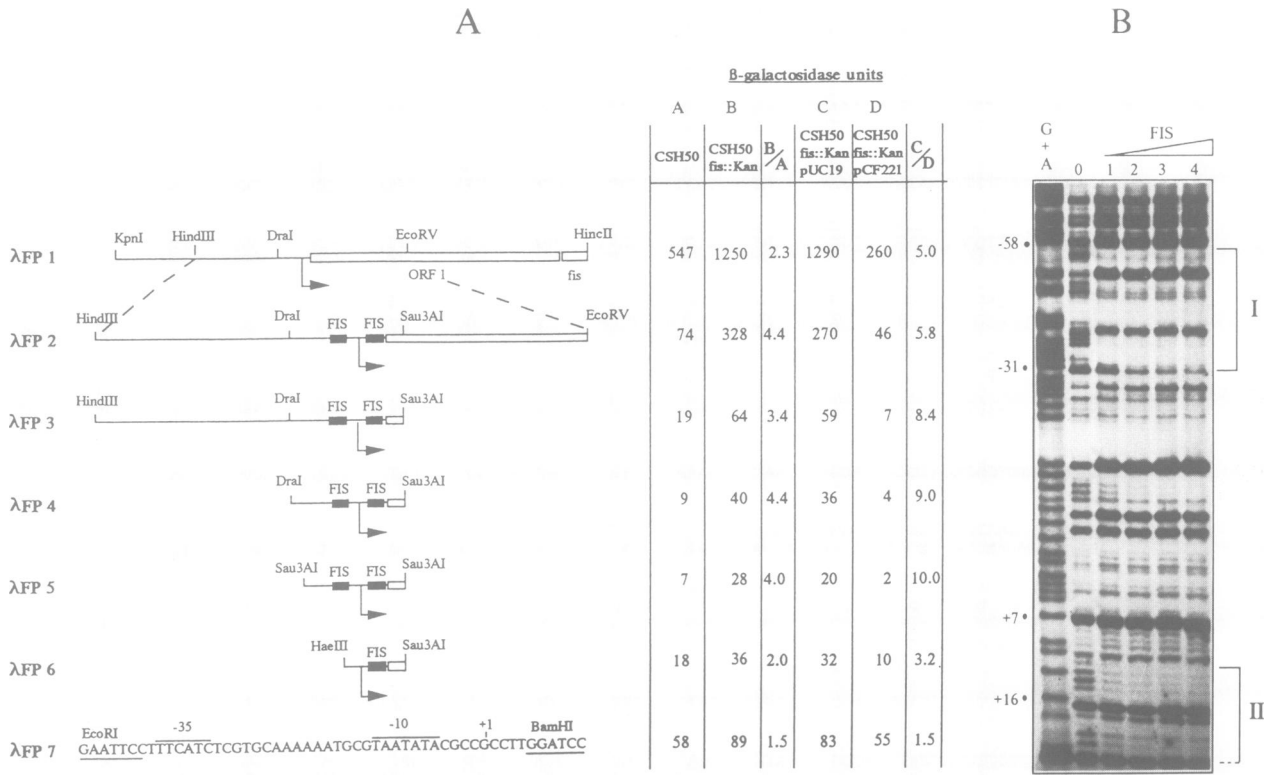

Fig. 3. Deletion analysis of the *fis* promoter and binding of FIS to the promoter. (**A**) On the left the parts of the *fis* operon fused to *lac*Z which are present in respective single copy λ lysogens are shown. FIS binding sites are indicated by black bars. On the right β-galactosidase units expressed in different genetic backgrounds are listed. (**B**) A 625 bp *Msp*I–*Eco*RV fragment 5′ labelled at the *Eco*RV end was incubated with increasing amounts of purified FIS protein and treated with limited amounts of DNase I as described in Materials and methods. 0, no protein was added; 1, 10 ng; 2, 20 ng; 3, 40 ng; 4, 80 ng FIS were added. G + A is a Maxam–Gilbert sequencing reaction of the same fragment. Regions protected by FIS are indicated by brackets, numbers refer to positions in the sequence according to Figure 2.

sequential deletions of sequences 5′ (λFP3-λFP6) or 3′ (λFP7) to the promoter was not affected by FIS.

Since the promoter fragments in λFP3 to λFP6 are fused to *lac*Z at identical sites in the vector the observed differences in promoter strength must be due to sequence context. It is conceivable that upstream sequences and in particular the short AT blocks provide for natural bends in the DNA which affect the downstream promoter as has been shown for other operons (Bracco *et al.*, 1989; Leirmo and Gourse, 1991). The 2- to 3-fold higher activity of the minimal promoter on λFP7 compared with promoters on λFP5 and λFP6 could either reflect differences in mRNA stability caused by different 3′ ends or again may reflect effects of sequence context on promoter activity.

Taken together these results show that FIS negatively autoregulates its own transcription as has been suggested before (Koch *et al.*, 1991) and links this effect to the presence of FIS binding sites I and II in the *fis* promoter. When plasmids carrying *fis*–*lac*Z fusions were introduced into strains lacking IHF no effect on promoter activity could be detected. This suggests that IHF does not participate in the regulation of *fis* synthesis although binding to a *fis* promoter fragment could be demonstrated by gel retention analysis (not shown).

Growth phase regulation of the minimal fis promoter

To investigate how the minimal *fis* promoter on λFP7 (Figure 3) is controlled, RNA was prepared from CSH50(λFP7) at various times after dilution of an overnight culture into fresh medium. For comparison RNA was prepared from CSH50(λtetP2) grown under the same conditions. In λtetP2 the constitutive tetracycline P2 promoter (Stüber and Bujard, 1981) is fused to *lac*Z. Northern blots were probed with a fragment of *lac*Z (Figure 4). For the tetP2 promoter a continuous increase of the fusion mRNA was observed with time (Figure 4A). The minimal *fis* promoter (Figure 4B), on the other hand, showed the same pattern of growth phase regulation as the full size *fis* promoter (Figure 1A). In stationary cells (Figure 4B, ON) no *fis*–*lac*Z mRNA was detected while a burst of expression occurred between 5 and 15 min after nutritional upshift. Upon further growth the amount of *fis*–*lac*Z mRNA declined. To get an idea which sequences of the minimal *fis* promoter are responsible for the observed expression pattern we generated three variants of the minimal promoter which are shown in Figure 5. In pFP8 the −35 region was mutated to the consensus −35 region, in pFP9 the −10 region was changed from TAATAT to TAATAG and in pFP10 sequences between −10 and the transcription start site were exchanged for the corresponding sequences from the β-lactamase (*bla*) promoter. The −10 mutation, which changes one of the most conserved residues in the consensus sequence (Harley and Reynolds, 1987) reduced promoter activity to background levels. The mutations in pFP8, on the other hand, led to a 4-fold increase in β-galactosidase activity compared with the wild-type promoter on pFP7. The substitution of *fis* sequences by *bla* sequences in pFP10 increased β-galactosidase activity ~5-fold suggesting

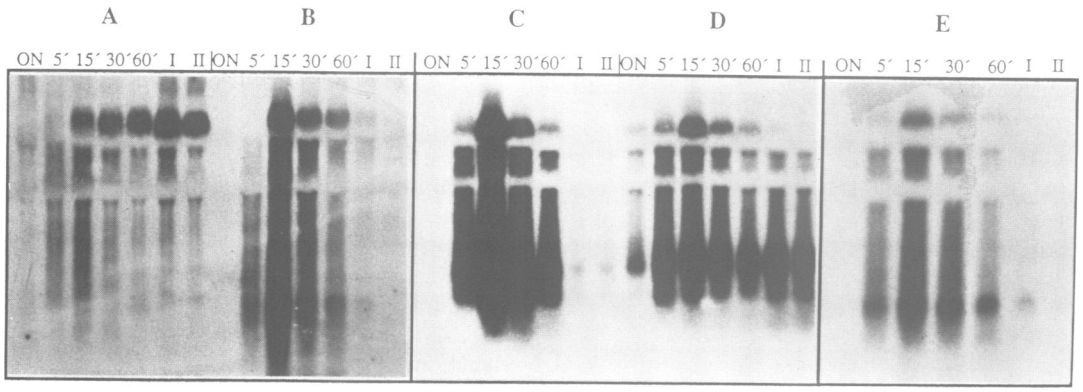

Fig. 4. Northern analysis of transcripts originating from the minimal *fis* promoter and control promoters. RNA was isolated at the indicated times (see legend to Figure 1) after 15-fold dilution of the overnight cultures in dYT from (**A**) CSH50(λP2*tet*); (**B**) CSH50(λFP7); (**C**) CSH50(λFP8); (**D**) CSH50 (λFP10) and (**E**) CSH50(λP1*rrn*B). Identical amounts of RNA were loaded in each slot. Northern analysis was performed with a labelled *lac*Z probe isolated as 900 bp *Eco*RI−*Cla*I fragment from pRS415.

		β-galactosidase units
	EcoRI -35 -10 BamHI	
pFP7	GAATTCCTTTCATCTCGTGCAAAAAATGCGTAATATACGCCGCCTTGGATCC	**1800**
pFP8	GAATTCCTTTGACATCGTGCAAAAAATGCGTAATATACGCCGCCTTGGATCC	**8000**
pFP9	GAATTCCTTTCATCTCGTGCAAAAAATGCGTAATAGACGCCGCCTTGGATCC	**40**
pFP10	GAATTCCTTTCATCTCGTGCAAAAAATGCGTAATATAACCCTGATAGGATCC	**9700**
		pRS415 **16**

Fig. 5. Activity of the minimal *fis* promoter and of promoter mutants. Mutations are outlined. β-galactosidase units were determined from overnight cultures of CSH50 harbouring the respective plasmids.

that a GC motif downstream of the −10 region affects the strength or the regulation of the *fis* promoter. To discriminate between these possibilities we examined the transcription pattern of λFP10 and λFP8 during a single growth cycle on Northern blots. The mutation in the −35 region led to increased transcription while the growth phase regulation of this mutant promoter showed the same pattern as the wild-type promoter (Figure 4C). In contrast, the mutation in the GC motif on λFP10 had no obvious effect on promoter strength but led to an altered regulation pattern. This promoter showed significant activity in late logarithmic phase and even in stationary phase cells (Figure 4D). Therefore the increased β-galactosidase activity observed with pFP10 (Figure 5) is not due to increased promoter strength. Instead it is the prolonged expression during the growth cycle which leads to accumulation of higher β-galactosidase levels in overnight cultures. GC-rich sequence motifs, or discriminator sequences (Travers, 1980), between the −10 region and the start site of transcription (Figure 5) are a characteristic feature of stable RNA promoters which are subject to stringent control (Cashel and Rudd, 1987; Zacharias *et al.*, 1989).

The fis promoter is stringently controlled

To analyse further the role of the GC-rich motif in the *fis* promoter we compared the minimal *fis* promoter with the *rrn*B P1 promoter which is known to be stringently controlled. The *rrn*B P1 promoter lacking upstream sequences was fused to *lac*Z and introduced as a single copy gene fusion on λ*rrn*B-P1 in CSH50. When RNA from this fusion gene (Figure 4E) was analysed in parallel with RNA from the minimal *fis* promoter on λFP7 (Figure 4B) we found a very similar expression profile for both promoters. This suggested that the *fis* promoter may be subject to stringent as well as growth rate control (Gallant, 1979; Lamond and Travers, 1985b; Gausing, 1977; Nierlich, 1978), a common feature of promoters directing stable RNA synthesis (Nomura *et al.*, 1984). To investigate this possibility further we have added chloramphenicol (Cm) to CSH50 cells in late logarithmic phase, at a stage where very little *fis* RNA is present. Chloramphenicol addition to starved stringent cells has been shown to result in the abrupt disappearance of ppGpp due to the accumulation of aminoacylated tRNAs when protein synthesis is inhibited (Kaplan *et al.*, 1973; Gallant, 1979). As a result promoter

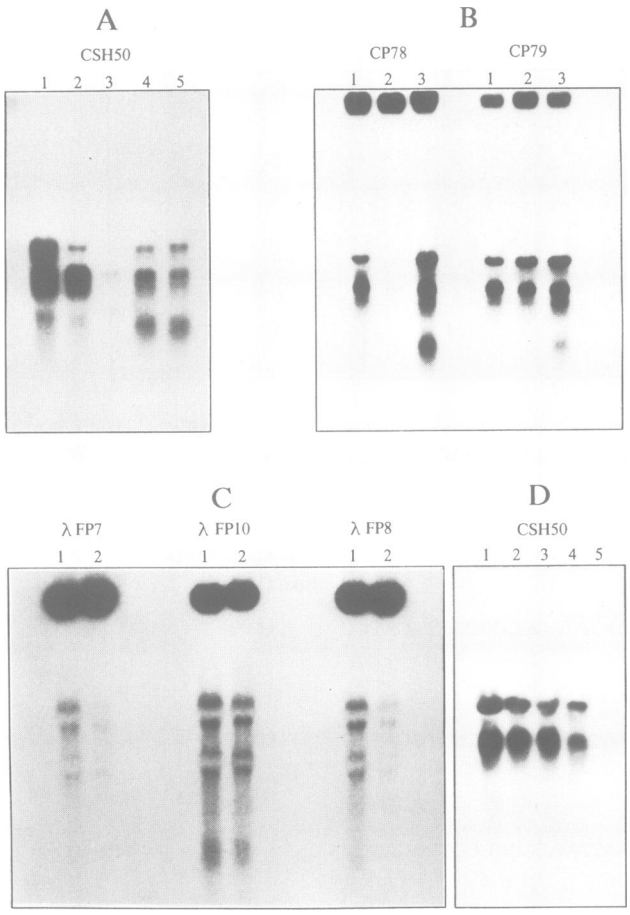

Fig. 6. Stringent control of *fis* expression demonstrated by Northern analysis. (**A**) An overnight culture of CSH50 in dYT was diluted 1:15 in dYT. RNA was prepared at 15 min (lane 1), 60 min (lane 2) and 120 min (lane 3). At 120 min chloramphenicol was added to 200 μg/ml and 10 and 60 min later RNA was isolated (lanes 4 and 5). (**B**) CP78 and CP79 were grown in modified Hershey's medium overnight and diluted 50-fold in the same medium. RNA was prepared after two doublings (lanes 1). At this time valine was added at 500 μg/ml and RNA was isolated 10 min later (lanes 2). At this time point chloramphenicol was added to 200 μg/ml and RNA was isolated 10 min later (lanes 3). (**C**) CSH50 lysogenic for the phages indicated was grown as in (B). RNA was prepared 15 min after nutritional upshift (lanes 1), stringent response was induced with valine as in (B) and 10 min later RNA was isolated (lanes 2). (**D**) An overnight culture of CSH50 was diluted 1:100 in dYT to an initial cell density of 4 Klett units. RNA was prepared after 100 min (10 Klett units, lane 1), after 3 h (30 Klett units, lane 2), after 3.7 h (70 Klett units, lane 3), after 4.5 h (100 Klett units, lane 4) and after 7 h (300 Klett units, lane 5). Northern blots were probed with a *fis* fragment (see legend to Figure 1) in (A), (B) and (D) and an *Eco*RI–*Cla*I fragment originating from *lac*Z of pRS415 in (C), respectively. The largest signal in (B) and (C) represents chromosomal DNA, in (A) and (D) this portion of the gel has been cut off.

activity of stringently controlled promoters is restored (Sokawa and Sokawa, 1978; Sarmientos *et al.*, 1983). RNA was isolated before and at 0, 10 and 60 min after addition of chloramphenicol. Northern analysis revealed that Cm addition restored activity of the *fis* promoter (Figure 6A, compare lane 3 with lanes 4 and 5). Next we determined the response of the *fis* promoter to amino acid starvation which induces the stringent response. Overnight cultures of strains CP78 and the isogenic *rel*A derivative CP79 in modified Hershey's medium (see Gourse *et al.*, 1983) were diluted into the same medium and grown to 2×10^8 cells per ml. At this time point a sample was removed for RNA analysis (Figure 6B, CP78, 1 and CP79, 1). At the same time valine was added at 500 μg/ml and incubation continued. Valine represses the isoleucine-valine operon which leads to isoleucine starvation (Lamond and Travers, 1985a; Gourse *et al.*, 1983). Ten minutes later samples were removed for RNA analysis (Figure 6B, CP78, 2 and CP79, 2). To the remaining cultures chloramphenicol was added to reactivate promoters shut off due to starvation and 10 min later RNA was prepared (Figure 6B, CP78, 3 and CP79, 3). Amino acid starvation led to a sharp drop in the amount of *fis* mRNA in CP78. After chloramphenicol treatment *fis* mRNA reappeared. In the *rel*A strain CP79, on the other hand, amino acid starvation did not affect the amount of *fis* mRNA.

To demonstrate that the GC motif in the *fis* promoter is responsible for the stringent control we have compared the mutant *fis* promoters on λFP8 and λFP10 with the wild-type *fis* promoter on λFP7. CSH50 harbouring the respective prophages were grown in modified Hershey's medium and subjected to the same amino acid starvation regime as described above. RNA was isolated before (Figure 6C, lanes 1) and 10 min after (Figure 6C, lanes 2) the addition of valine to the respective cultures. While the wild-type promoter (λFP7) and the promoter with improved −35 region (λFP8) were both shut off upon amino acid starvation the mutant promoter lacking the GC motif (λFP10) was no longer subject to stringent control. Since it has been shown for other stringently controlled promoters that they are active during exponential growth we have re-examined the rapid decline of *fis* synthesis observed in the other experiments. To this end we measured *fis* expression in CSH50 cells inoculated from an overnight culture into fresh medium at low cell density (1:100 dilution compared with the 1:30 or 1:15 dilutions used in previous experiments). RNA was isolated at four different time points during exponential growth and from stationary phase cells (Figure 6D). While there was no *fis* mRNA detectable in stationary cells, the amount of *fis* mRNA during exponential growth showed a high constant level for several doublings over a period of at least 4 h. This demonstrates that the *fis* promoter is active during exponential growth.

Discussion

The experiments presented have shown that the *fis* gene of *E.coli* is part of an operon containing one additional ORF. The *fis* operon is transcriptionally regulated by upstream sequences and by FIS. In addition, transcription is subject to stringent control.

Autoregulation

A comparison of *fis* promoter–*lac*Z fusions in *fis*$^-$ and *fis*$^+$ backgrounds revealed that FIS has a negative effect on its own transcription. This repression is ~4-fold and is independent of differences in absolute promoter strength of the different constructs analysed. The effect of FIS on its own regulation is directly linked to the presence of the two FIS binding sites mapped by footprint analysis (Figure 3B). When FIS is provided from a multicopy plasmid repression by FIS is increased up to a factor of 10 in constructs with both FIS binding sites whereas the minimal promoter is not affected. This shows that the *fis* promoter responds to differences in FIS levels. Autoregulation by FIS is the first

example described where FIS has a negative effect. In the Gin and Hin site specific recombination systems FIS enhances site specific inversion of DNA segments. FIS binding to the phage λ attR site stimulates the excision reaction and FIS binding to the upstream activator sequences of stable RNA operons activates transcription. The transcription activation of stable RNA synthesis is mediated by specific binding of FIS to the upstream activating sequences (UASs) of stable RNA promoters (Nilsson *et al.*, 1990; Ross *et al.*, 1990). In the *rrn*B P1 promoter FIS binds to three upstream sites and increases promoter activity between 20- and 30-fold *in vivo* and *in vitro* (Gourse *et al.*, 1986; Gaal *et al.*, 1989; Ross *et al.*, 1990). Since we have shown that *fis* transcription is negatively autoregulated, FIS is obviously able to act as activator or repressor depending on the position of binding sites relative to the start of transcription. The FIS binding sites in the *rrn*B P1 upstream sequence are centred at positions −71, −102 and −143 relative to the start of transcription (Ross *et al.*, 1990). In the *fis* promoter the FIS binding sites lie at different positions, centred at −42 (site I) and at +27 (site II) (Figure 2). The FIS binding site II occupies a position where in the *trp* and *lac* operons repressor binding sites were found (Bennett *et al.*, 1976; Reznikoff *et al.*, 1974). The position of the FIS binding site I upstream of the *fis* −35 region is unusual for binding of negative effector molecules. However, both binding sites lie at positions where FIS binding might either directly compete with binding of RNA polymerase or cause structural alterations of the DNA which may interfere with initiation of transcription. For the *rrn*B P1 promoter two mechanisms have been suggested for how FIS could stimulate transcription. Since it has been shown that the UAS of this promoter shows 2- to 4-fold residual upstream activation (Leirmo and Gourse, 1991) in *fis*$^-$ strains and DNA fragments containing the UAS are bent (Bossi and Smith, 1984; Gourse *et al.*, 1986; Vijgenboom *et al.*, 1988), it is conceivable that FIS stabilizes such bends in DNA and by doing so enhances the activating properties of these sequences. Alternatively FIS could directly interact with RNA polymerase in a way comparable with CRP. The function of this activator protein which, like FIS, bends the DNA (Liu-Johnson *et al.*, 1986) strictly depends on the spacing of the CRP binding site and the start point of transcription (Gaston *et al.*, 1990).

Binding of FIS to its own promoter will depend on the intracellular FIS concentration. By sensing the amount of unbound FIS directly this negative feedback control allows to determine the occupancy of FIS binding sites in the chromosome independent of growth rate.

Growth phase control

We have shown that the *fis* promoter activity is tightly linked to growth phase and that promoter sequences between −36 and +6 are sufficient to confer this transcription pattern. Since this minimal promoter is no longer subject to autoregulation (Figure 3) we conclude that FIS does not affect growth phase dependence of transcription. This is also supported by Northern blot analysis of a *fis* promoter−*lac*Z fusion in a *fis*$^-$ background which shows a regulation pattern comparable with that in *fis*$^+$ backgrounds (not shown). The response of the *fis* promoter to nutritional upshift is very similar to that of the ribosomal promoter *rrn*B P1. The *rrn*B P1 promoter used in our experiments lacks the upstream activating sequences which contain the FIS binding sites. Therefore FIS cannot be involved in generating the transcription pattern of this promoter (Figure 4E). These observations led us to consider comparable mechanisms for *rrn*B and *fis* promoter regulation. The promoters for rRNA and tRNA genes are regulated in a way that allows rapid adaptation of transcription to changes in growth conditions: the rate of stable RNA synthesis is proportional to the square of the growth rate while mRNA synthesis increases linearly with increasing cell growth. This relation is termed growth rate control (Gausing, 1977; Nierlich, 1978). Amino acid starvation leads to rapid decrease of stable RNA transcription (stringent control). For both phenomena guanosine tetraphosphate (ppGpp) is discussed as effector molecule (Baracchini and Bremer, 1988; Travers *et al.*, 1986). The cellular level of ppGpp is inversely correlated to promoter activity (Galland, 1979; Lamond and Travers, 1985b). We provide three lines of evidence that the *fis* promoter is stringently controlled. First, the *fis* promoter is strongly repressed by amino acid starvation. Second, this response is *rel*A dependent. Third, the ability of the promoter to respond to amino acid starvation is linked to the presence of the GC-rich motif downstream of the −10 region. Further support for the involvement of ppGpp in the regulation of *fis* transcription is provided by experiments in which Cm was added to the cultures. Addition of Cm to cells grown to high cell densities which lack *fis* mRNA restores *fis* promoter activity (Figure 6A). A similar effect is observed for the stringently repressed *fis* promoter (Figure 6B, CP78). In this experiment addition of Cm restores *fis* promoter activity even under stringent conditions. These results can be explained by the disappearance of ppGpp due to tRNA aminoacylation when protein synthesis is inhibited (Kaplan *et al.*, 1973; Gallant, 1979). We have also observed that inoculation of Cm treated stationary phase cells in Cm containing medium leaves the initial burst of *fis* transcription unaffected (not shown). This result rules out the need for *de novo* protein synthesis for activation of *fis* transcription. The growth phase dependent increase and decrease in *fis* promoter activity can thus be interpreted in terms established for stable RNA promoters. The burst of *fis* transcription following inoculation in fresh medium can be compared with the reaction of ribosomal promoters to nutritional upshift which is known to be very fast (Dennis and Bremer, 1974). The shift from an overnight culture to fresh medium represents such a nutritional upshift. Whereas the precise mechanism of this adaptation is not yet clear, the fact that *de novo* protein synthesis is not required makes ppGpp a good candidate for mediating this transcriptional activation as well. The steep decrease in promoter activity following the initial burst can be interpreted as concerted response to growth rate and stringent control mediated by ppGpp levels. If this interpretation is correct, the observed transcription pattern should be affected by the initial cell density. If an overnight culture is used to inoculate medium at high cell density as in the experiments shown in Figures 1 and 4, decreasing growth rates should directly follow initiation of growth without an extended period of exponential growth. The high ppGpp level in the overnight culture cells should drop to low levels after inoculaton in fresh medium and then increase again to high levels in a relatively short time (30−60 min) due to the fact that cells begin to enter stationary phase again. If an overnight culture is diluted to

low cell densities, on the other hand, the extended period of exponential growth should lead to an extended period of *fis* promoter activity. This is exactly what we have observed (Figure 6D).

Physiological implications

The finding that *fis* transcription is subject to both stringent control and autoregulation has some interesting implications concerning the role of FIS in the cell.

fis is transcribed at high levels during exponential growth and thus the function of FIS cannot be restricted to conditions like outgrowth from stationary phase or shifts in growth conditions. The levels of FIS in the cell vary with growth phase and thus may serve as an indicator for environmental conditions. This may help to couple phage λ excision, tail fibre variation in phage Mu and Hin mediated phase variation to a specific physiological state of the cell.

Stable RNA promoters and the *fis* promoter show the same response to changes in growth conditions. Since FIS is an activator of stable RNA transcription this will lead to an amplification of the environmental signals which trigger activation and repression of stable RNA synthesis. In turn the response of stable RNA promoters to nutritional upshift will be accentuated by increased synthesis of the transcriptional activator FIS while stringent conditions on the other hand will lead to repression of both stable RNA transcription and activator synthesis at the same time. Such a regulatory circuit is ideally suited for a rapid and precise adaptation to physiological changes. The autoregulation by FIS allows sensing of the occupancy of FIS binding sites in the chromosome independent of growth rate. This sensor is able to respond to changes in translational efficiency and to protein turnover. This sensor should also allow the monitoring of the concentrations of other DNA binding proteins like H1 and HU which bind DNA non-specifically and because of this could affect the accessibility of FIS binding sites.

Stringent control and growth rate regulation are control mechanisms which act nearly exclusively on genes which are involved in ribosome function. As yet *fis* and *dnaA* (Chiaramello and Zyskind, 1990; Hansen *et al.*, 1991) are interesting exceptions. Since the function of the ORF cotranscribed with *fis* is not known it is tempting to speculate that there could be a connection with ribosome function or DNA metabolism.

Materials and methods

Strains and media

Bacterial strains used in this study were *E.coli* K12 derivatives. CSH50 (*ara* Δ(*lac pro*) *strA thi*; Miller, 1972); CSH50fis::Kan is a *fis*$^-$ derivative of CSH50 (Koch *et al.*, 1988); CP78 (*relA*$^+$) and CP79 (*relA*$^-$) (Fiil and Friesen, 1968) were kindly provided by M.Zacharias. If not otherwise indicated strains were grown in dYT medium (Miller, 1972). To induce stringent response cells were grown in modified Hershey's medium supplemented with 18 amino acids lacking isoleucine and valine (Gourse *et al.*, 1983) to cell densities indicated in each experiment. Valine was added to 500 μg/ml at the times indicated.

Molecular analyses

Restriction enzymes and other DNA modifying enzymes were obtained from commercial sources and used according to the manufacturer's instructions. FIS protein was purified from an overproducing strain as described (Choe *et al.*, 1989). Standard techniques for bacterial transformation, DNA cloning and Northern analysis followed Sambrook *et al.* (1989). DNA sequencing was done according to Sanger *et al.* (1977) using single stranded and double stranded plasmid DNA templates of various subclones. Sequences were determined for both strands.

For primer extension the protocol of Hultmark *et al.* (1986) was used with some modifications. 10 ng of a 50 bp *NcoI*−*HpaII* fragment 5′ end-labelled at the *HpaII* site with [γ-^{32}P]ATP was coprecipitated with 30 μg of total *E.coli* RNA and treated as described in the original protocol. The primer extension reaction was performed in 150 μl RT-buffer containing 100 μM deoxynucleoside triphosphates and 60 units of reverse transcriptase (Genofit).

DNase I footprints with purified FIS protein

Footprinting was performed with the 690 bp *HindIII*−*EcoRV* fragment of pCF222. Both 5′ ends were labelled with polynucleotide kinase. The labelled fragment was cut with *MspI* and the resulting mixture of fragments (22 bp *HindIII*−*MspI*, 43 bp *MspI* and 625 bp *MspI*−*EcoRV*) was used for footprinting. Binding reactions were done in 170 μl at 20°C in 25 mM Tris−HCl pH 7.5, 75 mM NaCl, 0.1 mM EDTA, 1 μg/ml calf thymus DNA, 30 μg/ml BSA. After incubating with FIS for 5 min 17 μl of a DNase I solution (6.3 μg/ml in 100 mM $MgCl_2$) were added. DNase I treatment was carried out for 2 min and terminated by addition of 5 μl tRNA, 25 μl of 3 M sodium acetate, 20 μl of 25 mM EDTA and 230 μl phenol/chloroform. After extraction with phenol/chloroform and ethanol precipitation the DNA was analysed on a 6% sequencing gel.

Plasmids and phages

pRS415 is a pBR322 derived plasmid carrying the complete *lac* operon without promoter (Simons *et al.*, 1987). It was used to generate promoter−*lacZ* fusions by cloning fragments into the *EcoRI*, *SmaI* or *BamHI* site preceding the *lacZ* gene. Promoter−*lacZ* fusions were transferred to λRS45 by homologous recombination as described (Simons *et al.*, 1987). Phage lysates were prepared and used to lysogenize either CSH50 or CSH50fis::Kan by selecting for lysogens expressing β-galactosidase. For each construct up to 10 independent lysogens were isolated, purified and assayed for β-galactosidase expression. Double or triple lysogens which produced twice or three times as much β-galactosidase as single lysogens were eliminated. λ2C2 (Kohara *et al.*, 1987) contains the *fis* gene and flanking regions and was kindly provided by K.Isono. pCF221 is a pUC19 derived plasmid expressing *fis* (Koch *et al.*, 1988). pMLB1034 contains a *lacZ* gene without translational start which is preceded by a polylinker region (Silhavy *et al.*, 1984).

pCF222 contains a 1.6 kb *KpnI* fragment of λ2C2 comprising sequences upstream of *fis* cloned into the corresponding site of pTZ19R (Pharmacia).

pCF223 is a derivative of pCF221 in which sequences extending from the *KpnI* site upstream of *fis* to a *KpnI* site in the polylinker were removed and substituted by the 1.6 kb *KpnI* fragment of pCF222. This restores the *fis* operon.

pFP1 contains a 1.8 kb *KpnI*−*HincII* of the *fis* operon which was reconstituted in pMLB1034 first by combining a *HincII*−*SmaI* fragment of pCF221 with a *SmaI*−*NruI* fragment of pCF222. The fragment was then isolated as *EcoRI*−*BamHI* fragment for cloning in the respective sites of pRS415.

pFP2 contains a 650 bp *HindIII*−*EcoRV* fragment of pCF222 cloned into the *SmaI* site of pRS415 after blunting the ends with T4 polymerase.

pFP3 contains a 440 bp *HindIII*−*Sau3A* fragment of pCF222 cloned into the *SmaI*−*BamHI* sites of pRS415 after blunting the *HindIII* end with T4 polymerase.

pFP4 contains a 150 bp *DraI*−*Sau3A* fragment of pCF222 cloned into the *SmaI*−*BamHI* sites of pSR415.

pFP5 contains a 136 bp *Sau3A* fragment of pCF222 cloned into the *BamHI* site of pSR415.

pFP6 contains a 106 bp *HaeIII*−*Sau3A* fragment of pCF222 cloned into the *SmaI*−*BamHI* sites of pRS415.

pFP7 contains a 52 bp *EcoRI*−*BamHI* fragment assembled from two complementary oligonucleotides covering position −35 to +6 of the *fis* promoter.

pFP8 is identical to pFP7 except for four changes (positions −28 to −31) in the −35 region.

pFP9 is identical to pFP7 except for one change at position 6.

pFP10 is identical to pFP7 except for nine changes between positions +5 and −4.

pP1*rrn*B contains sequences from position +1 and −40 of the *rrnB* P1 promoter. They were synthesized as *EcoRI*−*BamHI* fragment and cloned into the *EcoRI*−*BamHI* sites of pRS415.

pP2tet contains the 375 bp *EcoRI*−*BamHI* fragment of pBR322 cloned in the respective sites of pRS415.

A map or sequence of the promoter regions present on these plasmids is shown in Figures 3 or 5, respectively. The inserts in pFP4-10 were

sequenced as was the insert in pP1*rrn*B. The promoter–*lacZ* fusions were transferred to λRS45 as described above, the nomenclature used for recombinant λ phages follows the plasmid nomenclature, e.g. λFP1 contains the promoter–*lacZ* fusion of pFP1.

Assay for β-galactosidase

Overnight cultures in dYT were assayed for β-galactosidase activity following the protocol of Sadler and Novick (1965). β-galactosidase units were multiplied by 1000 to make them approximately equivalent to those of Miller (1972).

RNA isolation and Northern analysis

At time points indicated in each experiment samples of ~10^9 cells were taken, chilled on ice and pelleted by centrifugation. The pellet was resuspended in 0.6 ml LETS buffer (100 mM LiCl, 10 mM EDTA, 10 mM Tris–HCl pH 7.4, 0.2% SDS). After addition of 0.6 ml phenol/chloroform (1:1, equilibrated with LETS) the sample was vortexed for 1 min. After centrifugation for 5 min at 4000 r.p.m. 5 μl 5 M LiCl and 1 ml EtOH were added to 0.45 ml of supernatant and kept at −20°C for 2 h. The RNA precipitate was collected by centrifugation. For Northern analysis ~10 μg RNA per lane was separated on a 1% agarose gel containing formaldehyde and transferred to a nylon filter (Sambrook *et al.*, 1989). All autoradiographs shown have been exposed overnight.

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Note added in proof

The nucleotide sequence data reported in this paper have been deposited in the EMBL, GenBank and DDBJ nucleotide sequence databases under the accession number X62399 E.coli orf1.